Cytokine Production by Human PBMC detected by EAS

Human peripheral blood mononuclear cells (PBMC) were stimulated for 5 hours with phorbol ester (PMA) and ionomycin in the presence of metabolic inhibitors (brefeldin A and monensin). In some of the cultures cyclosporin A (CsA), an IL-2 inhibitor at the mRNA level, was included. After incubation, the cells were fixed, permeabilized, and stained with anti-IL-2 antibodies (shaded histograms) or control Ig (open histograms). The cells were processed either by a standard amplification procedure (indirect staining) or by enzymatic amplification staining (EAS).

The results demonstrate that EAS can resolve high and low IL-2 producing cells (a new finding), that EAS can detect IL-2 producing cells without the use of metabolic poisons (a new finding), and that the validity of the EAS procedure is verified by CsA inhibition. It should also be noted that culture in the absence of stimulation did not give any positive staining (data not shown).

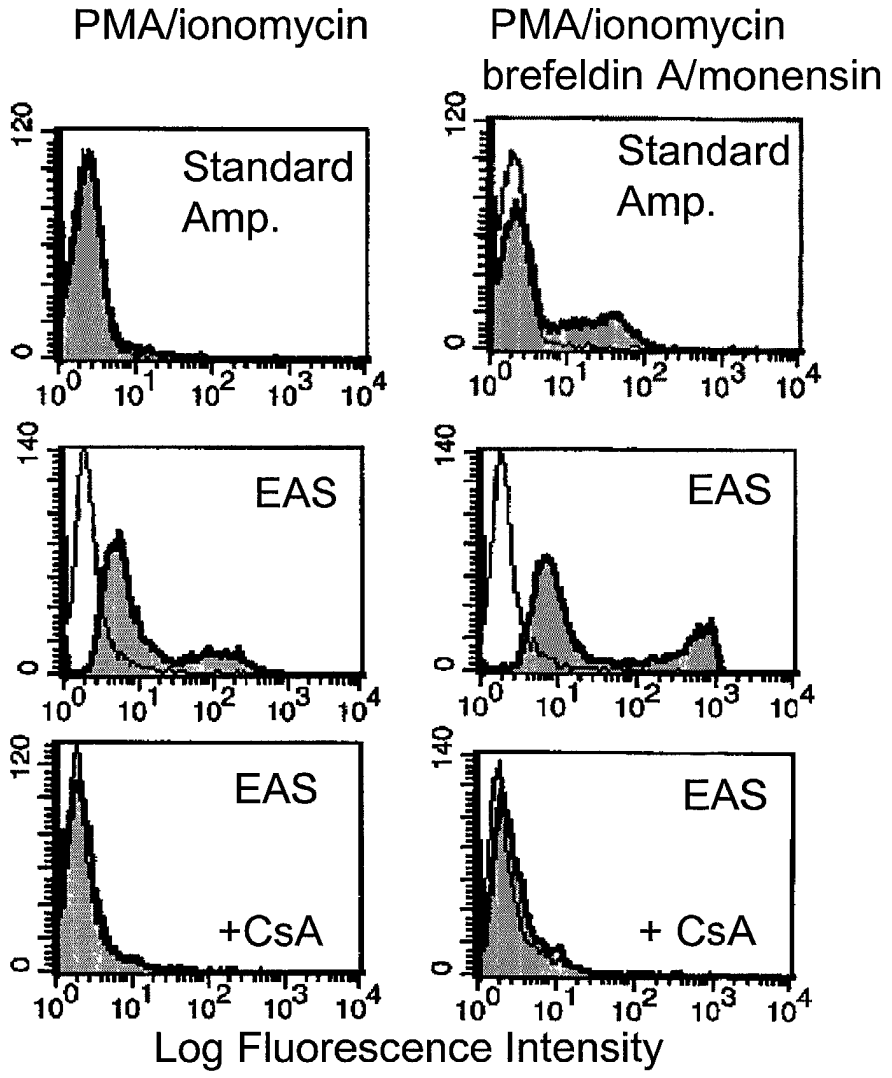

Fig. 1

Detection of bcl-2 by EAS

CEM cells were stained with anti-bcl-2 (filled histograms) or control Ig (open histograms. The cells were processed either by a standard amplification procedure or by EAS.

The results demonstrate that EAS gives a much greater separation of the specific signal from the background signal.

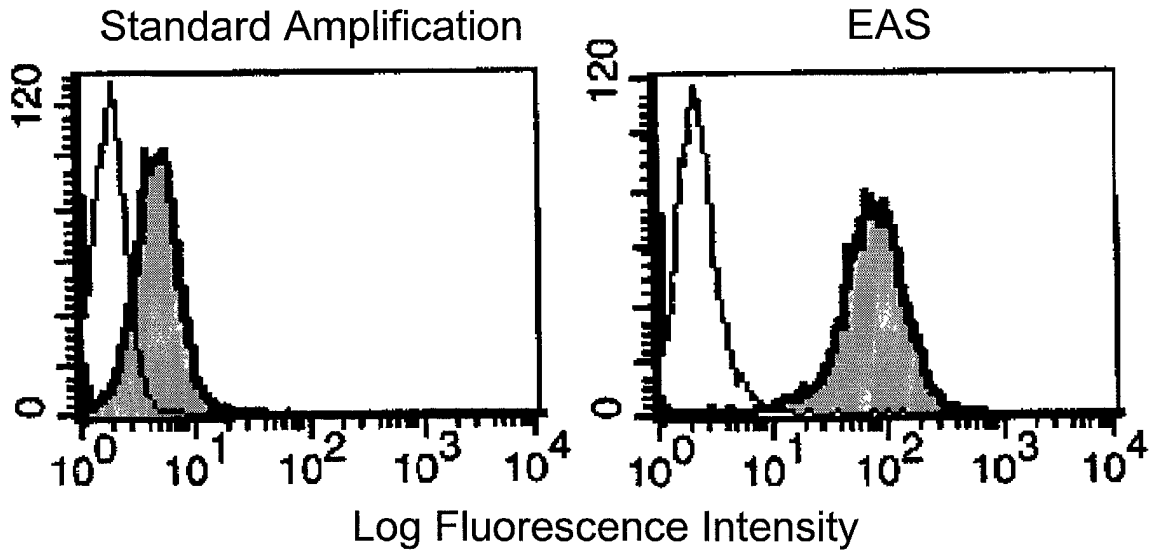

Fig. 2

Detection of Rb by EAS

CEM cells were stained with anti-Rb (filled histograms) or control Ig (open histograms. The cells were processed either by a standard procedure or by EAS.

The results demonstrate that EAS gives a much greater separation of the specific signal from the background signal.

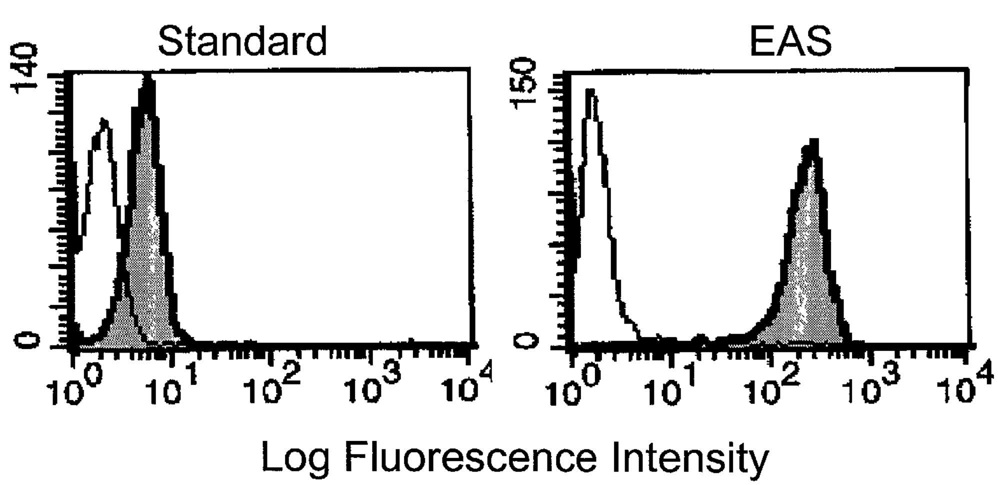

Fig. 3

Enzymatic Amplification Staining for Fluorescence In Situ Hybridization (FISH)

JY(LCL), Epstein-Barr Virus transformed B cells were probed with an antisense probe for EBER1 (open histogram) or with a nonsense probe (filled histogram). The probes were detected by FISH (left panel) or by enzymatic amplification of FISH (EAS FISH; right panel).

The results with EAS FISH show that EBER1 mRNA expression is heterogeneous with 2 major positive subpopulations with the most positive subpopulation demonstrating an approximate 100-fold enhancement in fluorescence intensity.

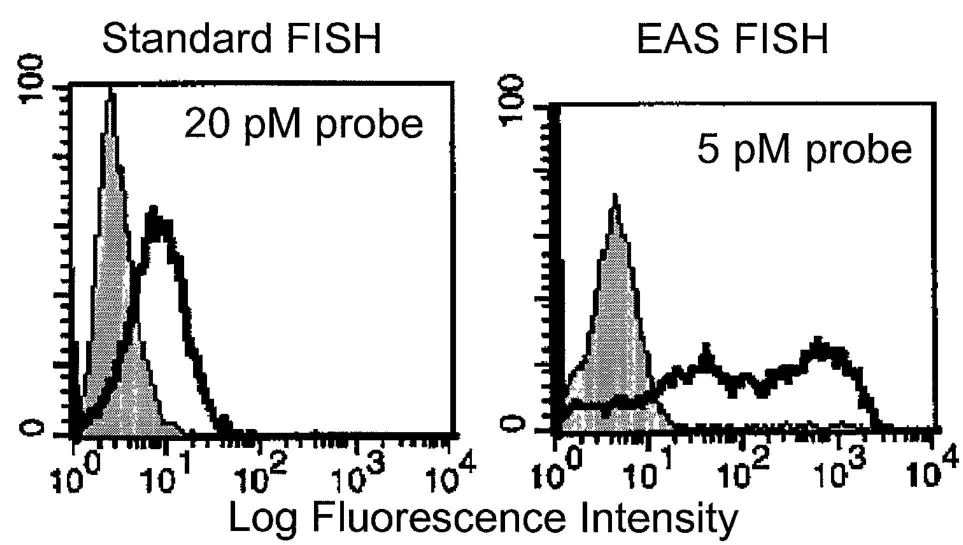

Fig. 4

EAS FISH for EBV EBER1 is Sensitive to Prior RNase treatment

JY(LCL), Epstein-Barr Virus transformed B cells, were probed with an antisense probe for EBER1 (filled histograms) or with a nonsense probe (open histograms). The probes were detected by fluorescence in situ hybridization (FISH) or by FISH with enzymatic amplification staining (EAS). The JY(LCL) cells shown in the right panel were treated with RNase prior to hybridization.

These results demonstrate that EAS FISH is sensitive to prior RNase treatment.

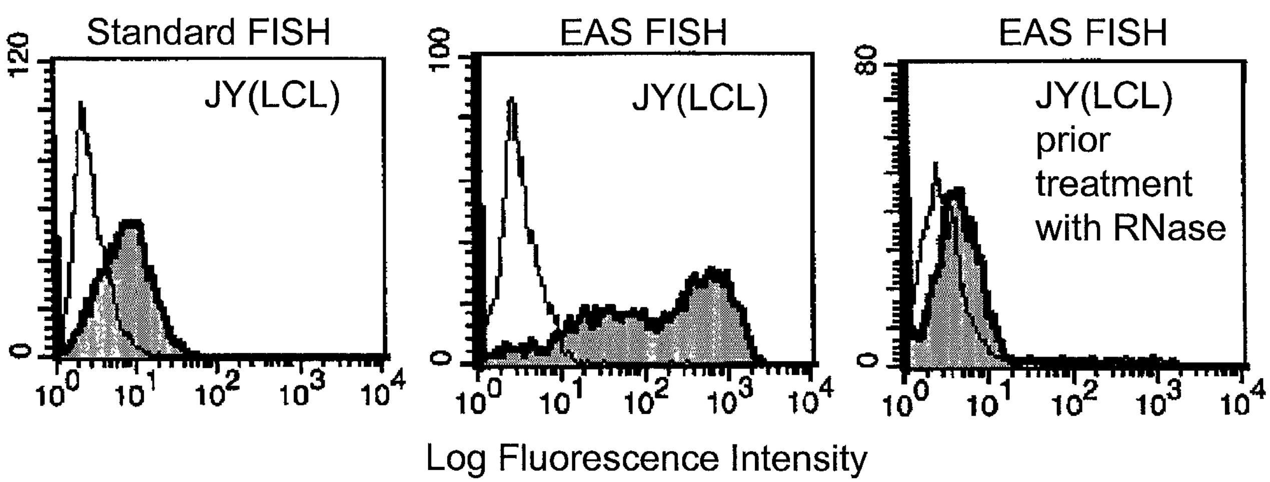

Fig. 5

Specificity of EAS FISH

EBER1 is an RNA species encoded by Epstein-Barr Virus (EBV). Various cell lines were probed with an antisense probe for EBER1 (filled histograms) or with a nonsense probe for EBER1 (open histograms). The probes were detected by fluorescence in situ hybridization (FISH) or by FISH with enzymatic amplification of the signal (EAS FISH). DK(LCL) and JY(LCL are EBV cells. BJAB is a B cell line that is not transformed by EBV and that thereby does not express any EBV mRNA. PBMC are peripheral blood mononuclear cells from a healthy volunteer.

The results demonstrate that EAS FISH demonstrates significantly greater signal for EBER1 than FISH does and that the signal seen with EAS FISH is found only in cells that are transformed by EBV.

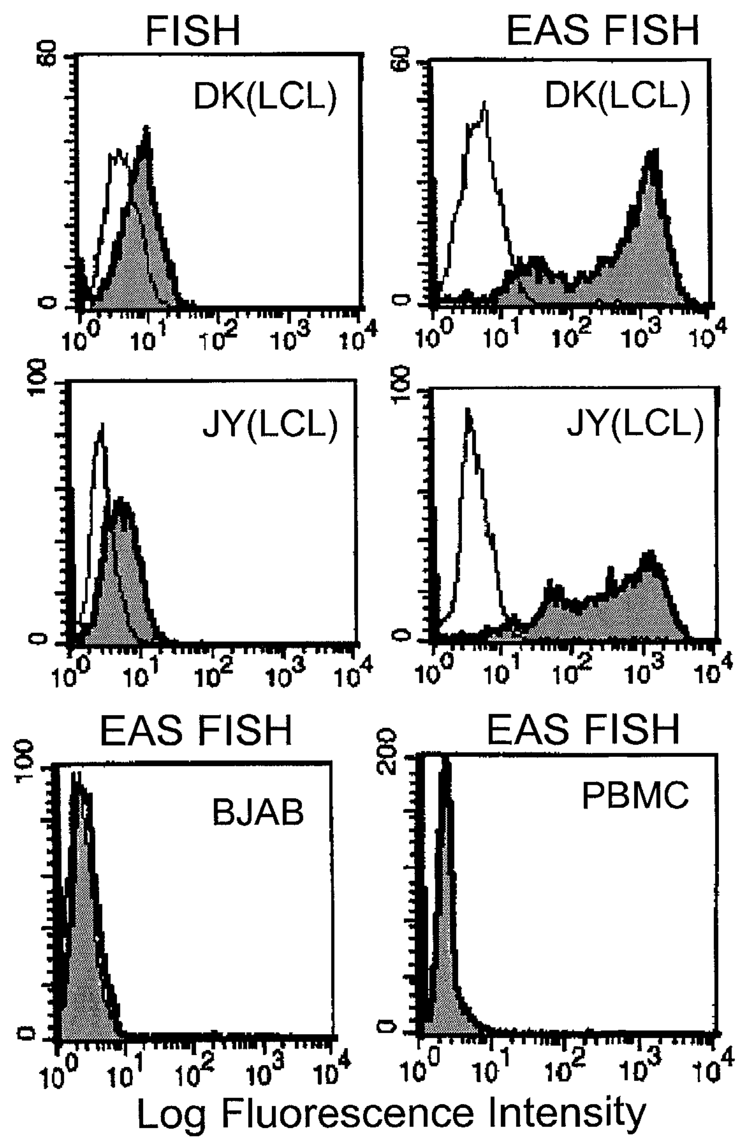

Fig. 6